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03 Biotechnology and its Applications

1. Introduction :

- Biotechnology, essentially deals with industrial scale production of biopharmaceuticals and biologicals using genetically modified microbes, fungi, plants and animals.
- The applications of biotechnology include therapeutics, diagnostics, genetically modified crops for agriculture, processed food, bioremediation, waste treatment, and energy production.
  Three critical research areas of biotechnology are:
  (a) Providing the **best catalyst** in the form of improved organism usually a microbe or pure enzyme.
  (b) Creating **optimal conditions** through engineering for a catalyst to act, and
  (c) Downstream processing technologies to **purify** the protein/organic compound.

2. Biotechnological Application in Agriculture :

Let us take a look at the three options that can be thought for increasing food production.

Increased Food Production :

I. **Agro-chemical based agriculture :** Based on **chemical fertilizers** and **chemical pesticides**. Results into increased production but quality decreases.
II. **Organic farming/agriculture :** **Chemical free** agriculture. Based on biofertilizers and biopesticides.
III. **Genetically engineered crop-based agriculture :** For this, desired gene is transferred in plant. Now these plants are called as **Genetic Modified (GM) / Transgenic plant/Tailor-made plants.**

- The Green Revolution succeeded in tripling the food supply but yet it was not enough to feed the growing human population.
- Increased yields have partly been due to the use of improved crop varieties, but mainly due to the use of better management practices and use of agrochemicals (fertilisers and pesticides).
- However, for farmers in the developing world, agrochemicals are often too expensive, and further increases in yield with existing varieties are not possible using conventional breeding.
- Is there any alternative path that our understanding of genetics can show so that farmers may obtain maximum yield from their fields? Is there a way to minimise the use of fertilisers and chemicals so that their harmful effects on the environment are reduced? Use of genetically modified crops is a possible solution.
- Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called **Genetically Modified Organisms (GMO).**

In addition to these uses, GM has been used to create **tailor-made plants** to supply alternative resources to industries, in the form of starches, fuels and pharmaceuticals.

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Use of Genetic modified (GM) plants:

(a) Enhanced nutritional quality of food,
***e.g.*, golden rice**, i.e., Vitamin 'A' enriched rice. (In this rice, gene of β-carotene is transferred)

(b) Made crops more tolerant to **abiotic stresses** (cold, drought, salt, heat).

(c) Helped to reduce **post-harvest losses**.
e.g. *Flavr Savr* Tomato : Transgenic variety of Tomato –Pectin degrading enzyme polygalacturonase is inhibited in *Flavr Savr* Tomato, So that tomato variety remains fresh and retain flavour much longer. *Flavr Savr* Tomato was developed by **anti-sense technology**.

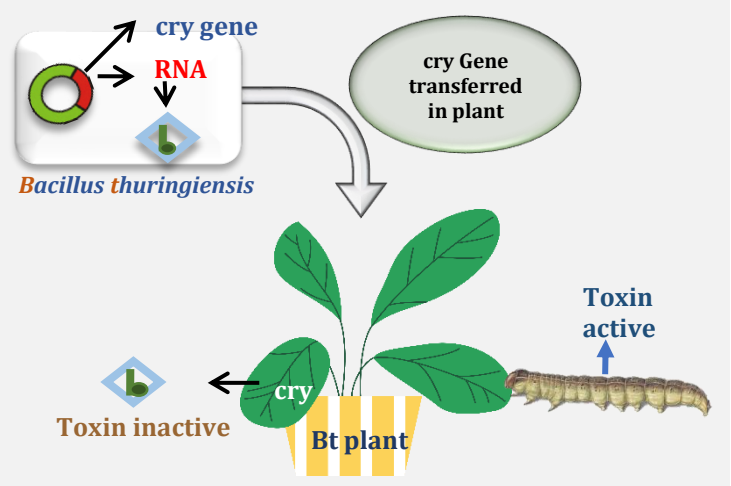
(d) Increased efficiency of mineral usage by plants (this **prevents early exhaustion** of fertility of soil).

(e) To produce biopharmaceutical products.
e.g. Production of Hirudin : **Hirudin** is a protein that prevents blood clotting. The gene encoding hirudin was chemically synthesized and transferred into ***Brassica napus***, where hirudin accumulates in seeds. The hirudin is purified and used as medicine.

(f) To produce herbicide resistant plant
e.g. First transgenic plant was tobacco. It contains resistant gene against herbicide (**Glyphosate**).

(g) Pest-resistant crops: Reduced reliance on **chemical pesticides** (pest-resistant crops).

(A) Pest (Insects) Resistant Plant:



- Some of the applications of biotechnology in agriculture that we will study in detail are the production of pest resistant plants, which could decrease the amount of pesticide used.
- **Bt toxin** is produced by a bacterium called ***Bacillus thuringiensis*** (Bt for short).
- Bt toxin gene has been cloned from the bacteria and been expressed in plants to provide resistance to insects without the need for insecticides; in effect created a bio-pesticide. Examples are Bt cotton, Bt corn, rice, tomato, potato and soyabean etc.
- Some strains of *Bacillus thuringiensis* produce proteins that kill certain insects such as lepidopterans (tobacco budworm, armyworm), coleopterans (beetles) and dipterans (flies, mosquitoes).
- *B. thuringiensis* forms protein crystals during a particular phase of their growth. These crystals contain a toxic insecticidal protein. Actually, the Bt toxin protein exist as inactive *protoxins* but once an insect ingest the inactive toxin, it is converted into an active form of toxin due to the alkaline pH of the gut which solubilise the crystals.

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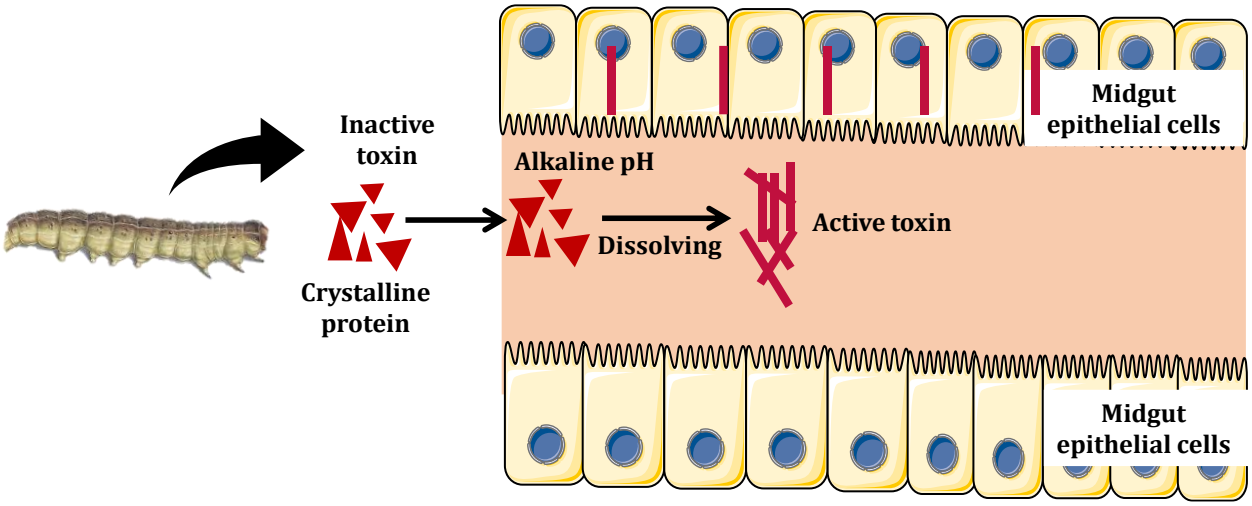
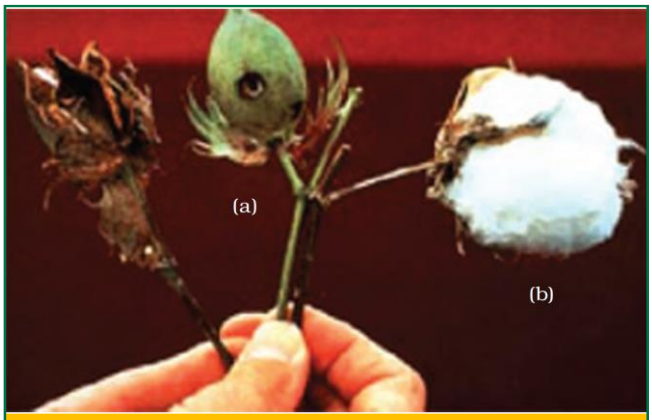


- The activated toxin binds to the surface of midgut epithelial cells and creates pores that cause cell swelling and lysis and eventually cause death of the insect.
- *Bacillus thuringiensis*, produces crystal [Cry] protein. This Cry protein is toxic to larvae of certain insects. Each Cry protein is toxic to a different group of insects.
- The gene encoding Cry protein is called "*cry gene*". This cry gene is isolated and transferred into several crops.
- However, gene symbol italics, ***e.g.*** *cry*. The first letter of the protein symbol, on the other hand, is always capital and the symbol is always written in roman letters. e.g. Cry.

Bt cotton :

- Specific Bt toxin genes were isolated from *Bacillus thuringiensis* and incorporated into the several crop plants such as cotton.
- The choice of genes depends upon the crop and the targeted pest, as most Bt toxins are insect-group specific.
- The toxin is coded by a gene ***cryIAc*** named cry. There are a number of them, for example, the proteins encoded by the genes ***cryIAc*** and ***cryIIAb*** control the **cotton bollworms**, that of ***cryIAb*** **controls corn borer**.



Cotton boll : (a) destroyed by bollworms; (b) a fully mature cotton boll

(B) Nematode (Pest) Resistant Plants:

- Several nematodes parasitise a wide variety of plants and animals including human beings. A nematode ***Meloidegyne incognitia*** infects the roots of **tobacco plants** and causes a great reduction in yield.
- A novel strategy was adopted to prevent this infestation which was based on the process of **RNA interference (RNAi).** RNAi takes place in all eukaryotic organisms as a method of **cellular defense**.

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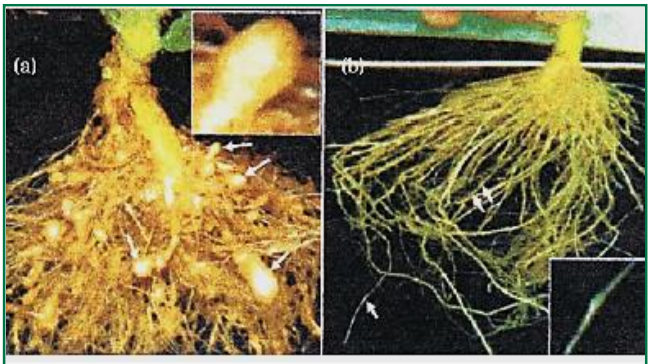
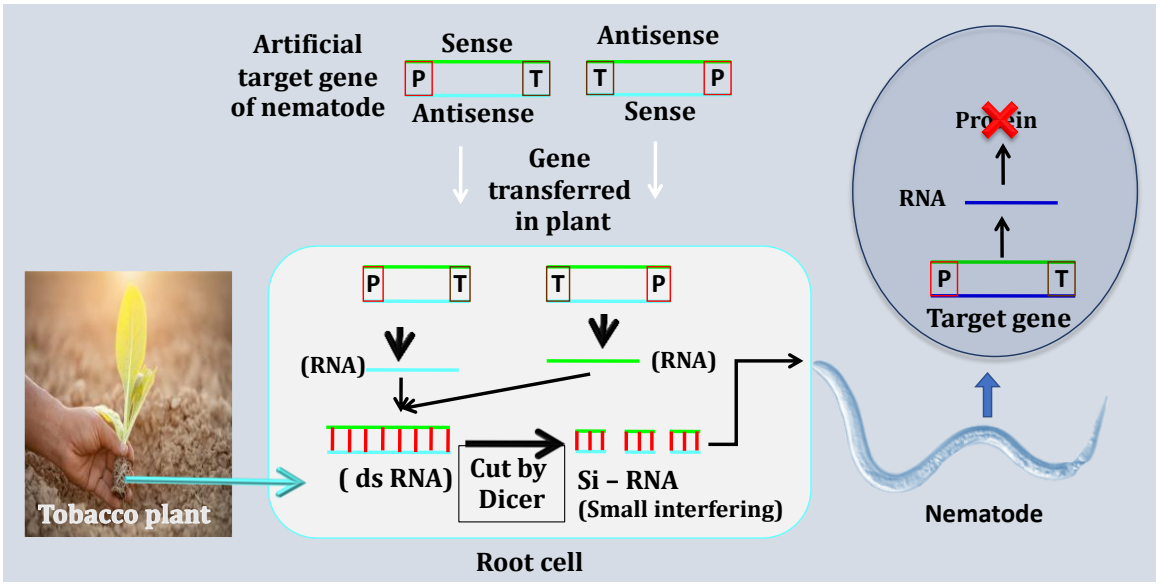
- This method involves silencing of a specific **mRNA** due to a complementary dsRNA molecule that binds to and prevents translation of the mRNA (silencing).
- The source of this complementary RNA could be from an infection by viruses having **RNA genomes** or mobile genetic elements (transposons) that replicate via an **RNA intermediate**.
- Using ***Agrobacterium*** vectors, nematode-specific genes were introduced into the host plant. The introduction of DNA was such that it produced both sense and anti-sense RNA in the host cells.
- These two RNA's being complementary to each other formed a double stranded (dsRNA) that initiated RNAi and thus, silenced the specific mRNA of the nematode.



Host plant-generated dsRNA triggers protection against nematode infestation :
(a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate infection of nematode but protected through novel mechanism.

The consequence was that the parasite could not survive in a transgenic host expressing specific interfering RNA. The transgenic plant therefore got itself protected from the parasite.



3. Biotechnological Applications in Medicine :

- The recombinant DNA technological processes have made immense impact in the area of healthcare by enabling mass production of safe and more effective therapeutic drugs. Further, the recombinant therapeutics do not induce unwanted immunological responses as is common in case of similar products isolated from non-human sources. At present, about 30 recombinant therapeutics have been approved for human-use the world over. In India, 12 of these are presently being marketed.

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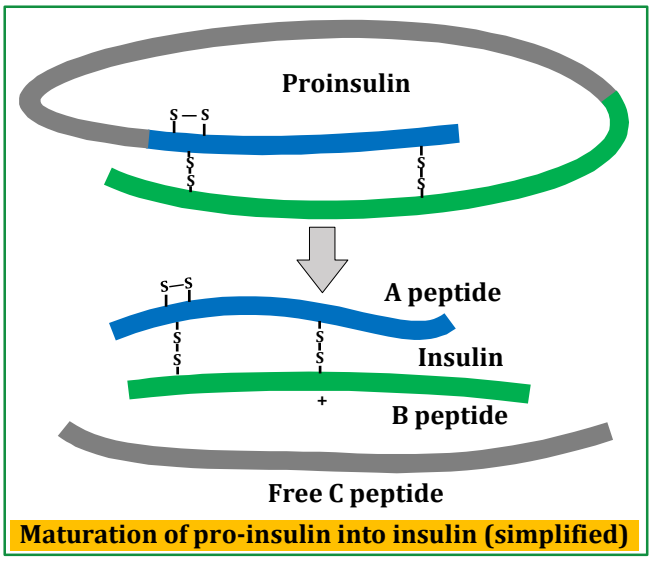
(A) Genetically Engineered Insulin :

- It is a proteinaceous hormone having 51 Amino acids arranged in two polypeptides A and B having 21 and 30 Amino Acids, respectively and joined by S-S disulphide bridges.
- **Sir Edward Albert Sharpey Schafer (1916)** was the first to note that diabetes of some persons was because of failure of some islands of pancreas to produce a substance which he called insulin (Derived from the latin word : insula meaning island).
- **Banting and best (1921)** were the first to isolate insulin from dog's pancreas and used it to cure diabetes in man.
- Insulin used for diabetes was earlier extracted from pancreas of slaughtered cattle and pigs.



Maturation of pro-insulin into insulin (simplified)

- Insulin from an animal source though caused some patients to develop allergy or other types of reactions to the foreign protein.
- Insulin consists of **two short polypeptide chains: chain A** and **chain B**, that are linked together by **disulphide** bridges.
- In mammals, including humans, insulin is synthesised as a pro-hormone (like a pro-enzyme, the pro-hormone also needs to be processed before it becomes a fully mature and functional hormone) which contains an extra stretch called the **C peptide**. This C peptide is not present in the mature insulin and is removed during maturation into insulin.
- The first genetically engineered insulin obtained by **recombinant DNA technique** with the help of ***E. coli*** was produced by the American firm, **Eli-Lilly** on July 5, 1983. It has been given the trade name **Humulin** and has been approved for clinical use.
- The main challenge for production of insulin using rDNA techniques was getting insulin assembled into a mature form. In 1983, **Eli Lilly** an American company prepared two DNA sequences corresponding to A and B, chains of human insulin and introduced them in plasmids of *E. coli* to produce insulin chains. Chains A and B were produced separately, extracted and combined by creating disulfide bonds to form human insulin.

(B) Gene Therapy :

- A new system of medicine gene therapy, may be used to treat some hereditary diseases such as SCID, haemophilia etc.
- Gene therapy is a collection of methods that allows correction of a gene defect that has been diagnosed in a child/embryo. Here genes are inserted into a person's cells and tissues to treat a disease.
- Correction of a genetic defect involves delivery of a normal gene into the individual or embryo to take over the function of and compensate for the **non-functional gene**.

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- The **first clinical gene therapy** was given in 1990 to a 4-year old girl with **adenosine deaminase (ADA) deficiency**. This enzyme is crucial for the immune system to function.
- The disorder is caused due to the deletion of the gene for adenosine deaminase. In some children ADA deficiency can be cured by **bone marrow transplantation**; in others it can be treated by **enzyme replacement therapy**, in which functional ADA is given to the patient by injection. But the problem with both of these approaches that they are not completely curative.

Steps :

- As a first step towards gene therapy, **lymphocytes** from the blood of the patient are grown in a culture outside the body.
- A functional ADA cDNA (using a **retroviral vector**) is then introduced into these lymphocytes, which are subsequently returned to the patient. However, as these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes.
- However, if the gene isolate from marrow cells producing ADA is introduced into cells at **early embryonic** stages, it could be a permanent cure.

(C) Medical Diagnosis of Disease (Molecular Diagnosis) :

- You know that for effective treatment of a disease, **early diagnosis** and understanding its **pathophysiology** is very important. Using conventional methods of diagnosis (**serum and urine analysis**, etc.) early detection is not possible.
- **Recombinant DNA technology**, **Polymerase Chain Reaction (PCR)** and **Enzyme Linked Immuno-Sorbent Assay (ELISA)** are some of the techniques that serve the purpose of early diagnosis.
- Presence of a pathogen (bacteria, viruses, etc.) is normally suspected only when the pathogen has produced a disease symptom.
- By this time the concentration of pathogen is already very high in the body. However, very low concentration of a bacteria or virus (at a time when the symptoms of the disease are not yet visible) can be detected by amplification of their nucleic acid by PCR.
- PCR is now routinely used to detect HIV in suspected AIDS patients. It is being used to detect mutations in genes in suspected cancer patients too. It is a powerful technique to identify many other genetic disorders.
- A single stranded DNA or RNA, tagged with a **radioactive molecule** (probe) is allowed to hybridise to its complementary DNA in a clone of cells followed by detection using autoradiography. The clone having the mutated gene will hence not appear on the photographic film, because the probe will not have complementarity with the mutated gene.
- ELISA is based on the principle of **antigen-antibody** interaction. Infection by pathogen can be detected by the presence of **antigens**(proteins, glycoproteins, etc.) or by detecting the **antibodies** synthesised against the pathogen.

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4. Transgenic Animals:

- Animals that have had their DNA manipulated to possess and express an **extra (foreign) gene** are known as **transgenic animals**. Transgenic rats, rabbits, pigs, sheep, cows and fish have been produced, although over 95% of all existing transgenic animals are **mice**.

(A) Normal Physiology and Development :

- Transgenic animals can be specifically designed to allow the study of how genes are regulated, and how they affect the normal functions of the body and its development, e.g., study of complex factors involved in growth such as insulin-like growth factor.
- By introducing genes from other species that alter the formation of this factor and studying the biological effects that result, information is obtained about the biological role of the factor in the body.

(B) Study of Disease :

- Many transgenic animals are designed to increase our understanding of how genes contribute to the development of disease. These are specially made to serve as **models for human diseases** so that investigation of new treatments for diseases is made possible. Today transgenic models exist for many human diseases such as **cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's.**

(C) Biological Products :

- Medicines required to treat certain human diseases can contain biological products, but such products are often expensive to make.
- Transgenic animals that produce useful biological products can be created by the introduction of the portion of DNA (or genes) which codes for a particular product such as **human protein (α-1-antitrypsin)** used to treat **emphysema**. Similar attempts are being made for treatment of **phenylketonuria** (PKU) and **cystic fibrosis**.
- In 1997, the first **transgenic cow**, **Rosie**, produced human protein-enriched milk (2.4 grams per litre). The milk contained the human **alpha-lactalbumin** and was nutritionally a more balanced product for human babies that natural cow-milk.

(D) Vaccine Safety :

- Transgenic mice are being developed for use in testing the safety of vaccines before they are used on humans.
- Transgenic mice are being used to test the safety of the polio vaccine. If successful and found to be reliable, they could replace the use of monkeys to test the safety of batches of the vaccine.

(E) Chemical Safety Testing :

- This is known as **toxicity/safety testing**. The procedure is the same as that used for testing toxicity of drugs.
- Transgenic animals are made that carry genes which make them more sensitive to toxic substances than non-transgenic animals. They are then exposed to the toxic substances and the effects studied. Toxicity testing in such animals will allow us to obtain results in less time.

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Applications of Recombinant DNA products

Medically useful recombinant products	Applications
Human insulin	Treatment of insulin - dependent diabetes
Human growth hormone	Replacement of missing hormone in short stature people.
Calcitonin	Treatment of rickets.
Chorionic gonadotropin	Treatment of infertility.
Blood clotting factor VIII/IX	Replacement of clotting factor missing in patients with Haemophilia A/B.
Tissue Plasminogen Activator (TPA)	Dissolving of blood clots after heart attacks and strokes.
Erythropoetin	Stimulation of the formation of erythrocytes (RBCs) for patients suffering from anaemia during dialysis or side effects of AIDS patients treated by drugs.
Platelet derived growth factor	Stimulation of wound healing
Interferon	Treatment of pathogenic viral infections, cancer
Interleukin	Enhancement of action of immune system
Vaccines	Prevention of infectious diseases such as hepatitis B, herpes, influenza, pertussis, meningitis, etc.

Application of Genetically Engineered Microbes

Microbes	Applications
Escherichia coli (gut bacterium)	Production of human insulin, human growth factor interferons, interleukin and so on.
Bacillus thuringiensis (soil bacterium)	Productions of endotoxin (Bt toxin), highly potent, safe and biodegradable insecticide for plant protection.
Rhizobium meliloti (bacterium)	Nitrogen fixation by incorporating "nif" gene in cereal crops.
Pseudomonas putida (bacterium)	Scavenging of oil spills by digesting hydrocarbons of crude oil.
Bacterial strains capable of accumulating heavy metal	Bioremediation (cleaning of pollutants in the environment).
Trichoderma (fungus)	Production of enzyme chitinases for biocontrol of fungal diseases in plants.

Transgenics and their potential applications

Transgenic organism	Useful applications
Bt Cotton	Pest resistance and high yield.
Flavr Savr Tomato	Increased shelf-life (delayed ripening) and better nutrient quality
Golden Rice	Vitamin A and Fe – rich
Cattles (cow, sheep, goat)	Therapeutic human proteins in their milk
Pig	Organ transplantation without risk of rejection

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5. Ethical Issues:

- The manipulation of living organisms by the human race cannot go on any further, without regulation. Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms.
- Going beyond the morality of such issues, the biological significance of such things is also important. Genetic modification of organisms can have **unpredictable results** when such organisms are introduced into the ecosystem.
- Therefore, the Indian Government has set up organisations such as **GEAC (Genetic Engineering Approval Committee)**, which will make decisions regarding the validity of GM research and the safety of introducing GM-organisms for public services.
- The modification/usage of living organisms for public services (as food and medicine sources, for example) has also created problems with patents granted for the same.
- GM crops are already in cultivation in U.S.A., Europe and several other countries. In India, some insect resistant cotton varieties expressing cry genes have reached the farmers fields. It has been argued that transgenic crops may be harmful to the environment. The two points :-
- **Firstly,** the transgene may be transferred through pollen from these crops to their wild relatives.
- **Secondly,** GM crops may pollute the environment.

6. Bio-Patent :

A patent is a right granted by a government to an inventor to prevent others from commercial use of his invention. A patent is granted for:

(a) An invention (including product)
(b) An improvement in an earlier invention
(c) The process of generating products and
(d) A concept or design.

- There is growing public anger that certain companies are being granted patents for products and technologies that make use of the genetic materials, plants and other biological resources that have long been identified, developed and used by farmers and indigenous people of a specific region/country.
- Rice is an important food grain, the presence of which goes back thousands of years in Asia's agricultural history. There are an estimated 200,000 varieties of rice in India alone. The diversity of rice in India is one of the richest in the world. Basmati rice is distinct for its unique aroma and flavour and 27 documented varieties of Basmati are grown in India. There is reference to Basmati in ancient texts, folklore and poetry, as it has been grown for centuries.
- In 1997, an American company got patent rights on Basmati rice through the US Patent and Trademark Office. This allowed the company to sell a 'new' variety of Basmati, in the US and abroad. This 'new' variety of Basmati had actually been derived from Indian farmer's varieties. Indian Basmati was crossed with semi-dwarf varieties and claimed as an invention or a novelty.
- The patent extends to functional equivalents, implying that other people selling Basmati rice could be restricted by the patent.
- Several attempts have also been made to patent uses, products and processes based on Indian traditional herbal medicines, ***e.g.***, turmeric, neem. If we are not vigilant and we do not immediately counter these patent applications, other countries/individuals may encash on our rich legacy and we may not be able to do anything about it.

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7. Biopiracy :

- **Biopiracy** is the term used to refer to the **use of bio-resources** by multinational companies and other organisations without proper authorisation from the countries and people concerned without compensatory payment.
- Most of the industrialised nations are rich financially but poor in biodiversity and traditional knowledge. In contrast the developing and the underdeveloped world is rich in biodiversity and traditional knowledge related to bio-resources. Traditional knowledge related to bio-resources can be exploited to develop modern applications and can also be used to save time, effort and expenditure during their commercialisation.
- There has been growing realisation of the injustice, inadequate compensation and benefit sharing between developed and developing countries. Therefore, some nations are developing laws to prevent such unauthorised exploitation of their bio-resources and traditional knowledge.
- The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill, that takes such issues into consideration, including patent terms emergency provisions and research and development initiative.

★ Golden Key Points ★

- First transgenic animal was mouse containing gene for growth hormone. This enlarged mouse was known as **Super mouse**.
- First introduced transgenic crop in India (2002) is **Bt-cotton**.
- **Charles Weismann** of university of Zurich, obtained **interferon** through recombinant *E. coli* (1980)
- Microbes have been engineered to produce Human Growth Hormone (**HGH**) for curing dwarfism.
- **Vaccines** which are produced by genetic engineering e.g. for Hepatitis-B and Herpes virus.
- Nitrogen fixation genes may be transferred from bacteria to the major food crops to boost food production without using expensive fertilizers.
- **Bioremediation:** In pollution control, microbes have been engineered to break up the crude oil spills. **Dr. Ananda Mohan Chakrabarty** introduced plasmids from different strains into a single cell of ***Pseudomonas putida*.** The result was new genetically engineered bacterium which would clean the oil spills called "**Superbug**" (Oil eating bug). He transferred four types of genes/plasmids in these bacteria. These are OCT, XYL, CAM & NAH.
- **Genetic modified food** – The food prepared from genetically modified crop[transgenic] is called genetically modified food or **G.M. Food**.

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BEGINNER'S BOX-1 BIOTECHNOLOGY APPLICATION TO BIO-PIRACY

1. Bt toxin kills the insect by:-
(1) Blocking the nerve conduction (2) Damaging the surface of trachea
(3) By creating pores in the tracheal system (4) By creating pores in the mid gut

2. Which is not an application of modern biotechnology?
(1) Production of humulin (2) Developing a DNA vaccine
(3) Gene therapy (4) Production of cheese and butter

3. Transgenic *Brassica napus* has been used for the synthesis of :-
(1) Hirudin (2) Heparin (3) Polgalacturonase (4) Cry protein

4. Transgenic tobacco plant was developed by the process of RNA interference, was resistant against the infection of :-
(1) Algae: *Scenedesmus* (2) Fungi: *Fusarium*
(3) Bacteria: *Bacillus thuringienesis* (4) Nematode: *Meloidegyne incognitia*

5. The first clinical gene therapy was given for treating :-
(1) Rheumatoid arthritis (2) Adenosine deaminase deficiency
(3) Diabetes (4) Chicken pox

6. Protein encoded gene cryIAb controls: -
(1) Cotton bollworm (2) Beetles (3) Corn borer (4) Flies

7. RNAi stands for: -
(1) RNA infection (2) RNA induction (3) RNA interference (4) RNA inhibition

8. ELISA Test is based on which interaction: -
(1) Antigen-antibody interaction (2) Antigen –RBC interaction
(3) Pathogen-RBC interaction (4) RBC-WBC interaction

9. The conventional method of diagnosis involves: -
(1) Urine analysis (2) ELISA (3) PCR (4) rDNA technology

10. Which of the following is used as probe?
(1) Single stranded DNA (2) dsDNA tagged with a radioactive molecule
(3) Single stranded RNA tagged (4) dsRNA

11. Maturation of proinsulin into insulin takes place after:-
(1) Joining of C peptide (2) Removal of C peptide
(3) Removal of disulphide bridge (4) Addition of disulphide bridge

12. A plant expressing a gene from another organism is :-
(1) Transgenic (2) Clone (3) Soma clonal variant (4) Transformed

BEGINNER'S BOX ANSWERS KEY

BEGINNER'S BOX-1	Que.	1	2	3	4	5	6	7	8	9	10
	Ans.	4	4	1	4	2	3	3	1	1	3
	Que.	11	12								
	Ans.	2	1								

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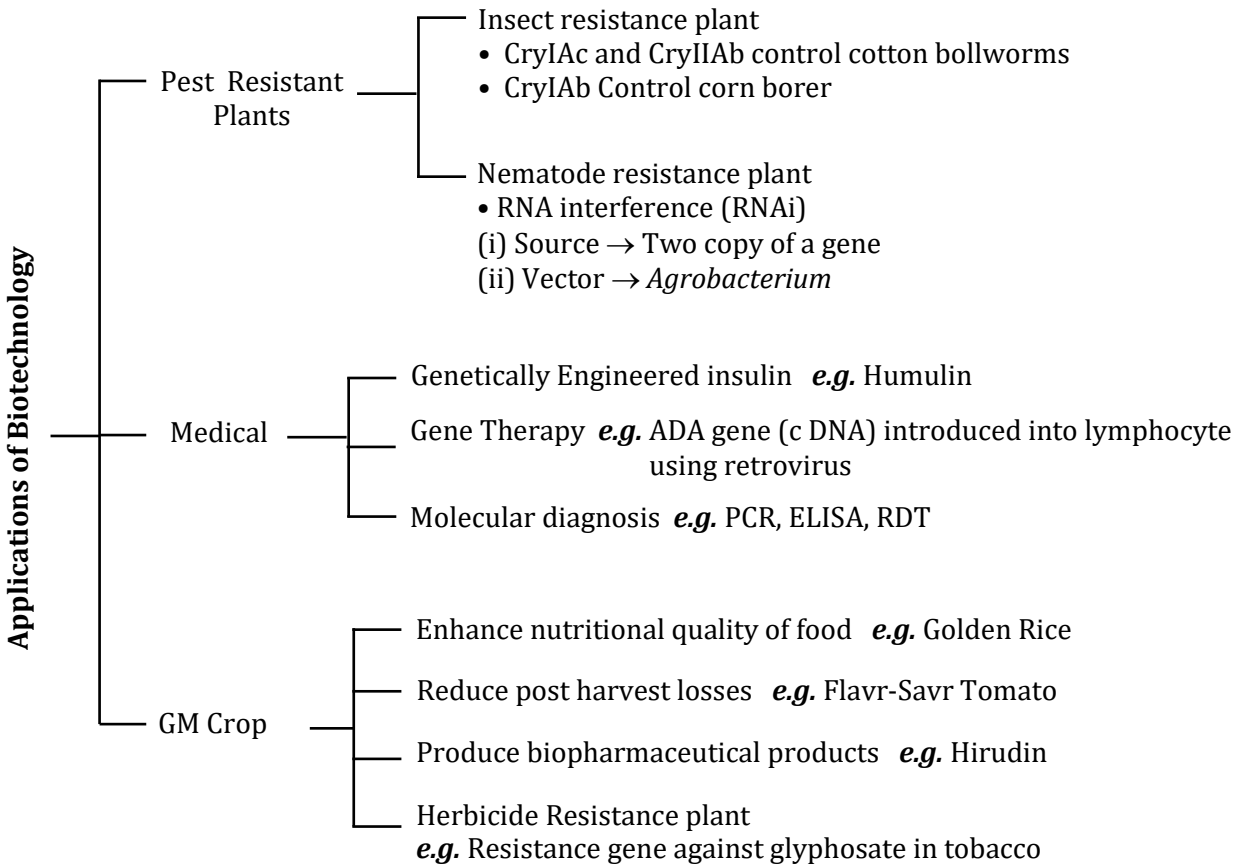
Applications of Biotechnology
Pest Resistant Plants
Insect resistance plant
• CryIAc and CryIIAb control cotton bollworms
• CryIAb Control corn borer
Nematode resistance plant
• RNA interference (RNAi)
(i) Source → Two copy of a gene
(ii) Vector → Agrobacterium
Medical
Genetically Engineered insulin e.g. Humulin
Gene Therapy e.g. ADA gene (c DNA) introduced into lymphocyte using retrovirus
Molecular diagnosis e.g. PCR, ELISA, RDT
GM Crop
Enhance nutritional quality of food e.g. Golden Rice
Reduce post harvest losses e.g. Flavr-Savr Tomato
Produce biopharmaceutical products e.g. Hirudin
Herbicide Resistance plant
e.g. Resistance gene against glyphosate in tobacco

Transgenic Animals

Study of Diseases
- Cancer
- Cystic fibrosis
- Rheumatoid arthritis
- Alzheimer

Vaccine Safety
- Transgenic mice are being developed for use in testing the safety of vaccines (such as-polio vaccine)

Biological Product
- α-1 antitrypsin used to treat Emphysema
- Rosie, produced human protein (alpha-lactalbumin) enriched milk